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Linking structure and function of microbial communities in rapid sand filters using molecular tools and bioenergetics

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Rapid sand filtration is a commonly used method for the production of potable water from groundwater. RSF can achieve near complete removal of nutrients in drinking water ensuring high biological stability as required for non-disinfected distribution systems and to meet the strict regulatory limits in the EU and Denmark. This method consists of aeration of anoxic groundwater followed by filtration through either a single filter, or pair of filters connected in series. While biological processes are believed to be solely responsible for ammonium removal, the removal of other common groundwater contaminants, methane, sulfide, iron and manganese, can occur by both physicochemical and biological processes, and the relative importance of biological oxidation is not clear. In order to gain a clearer perspective of the biological functionality of the filters, we examined the microbial communities in more than 15 RSFs in Denmark by 16S rRNA gene amplicon sequencing and qPCR. Total cell numbers average around  $5 \times 10^9$ /g of filter material. The microbial community compositions reveal a core community that emphasizes the importance of biological processes in contaminant removal in RSF. The communities comprise primarily nitrifiers (40%), along with methane, sulfide, and metal oxidizing bacteria, and a number of heterotrophs / bacteria of unknown function. *Nitrospira* spp. are the dominant organisms in filters and comprise both completely nitrifying comammox *Nitrospira* and nitrite-oxidizing taxa (18-55%). Furthermore, canonical ammonia oxidizing bacteria (AOB) are observed in all filters though at low abundance (<1-10%). While chemical data was collected at all examined filters, we performed detailed quantification of contaminants in select filters in an effort to relate the structure of the microbial community with the biological energy inputs. In a time series sampling, we measured methane, Fe(II), Fe(III), Mn(II), Mn(IV), nitrogen and sulfur species, and assimilable organic carbon (AOC) in the influent, effluent and between pre- and after-filters if present. Of particular interest is the amount of organic carbon serving as a biological substrate (AOC), as up to 40% of the microbial community consists of bacteria with unknown function, but the NVOC in the influent and effluents are typically not significantly different. Our results show that RSF communities are biodiverse and reflect the biological functions occurring in the filters. We show that the novel comammox *Nitrospira* are the dominant nitrifiers, along with nitrite oxidizing *Nitrospira* and canonical AOB and AOA. In addition, all filters harbour microbes associated with methane, sulfide, metal and organic carbon oxidation, emphasizing the importance of biological contaminant treatment in RSFs used for drinking water production.